Reference: *Biol. Bull.,* **150**: 491–499. (June, 1976)

HATCHING SIZE AND THE DISTRIBUTION OF NURSE EGGS AMONG PROSOBRANCH EMBRYOS [1]

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A prosobranch embryo that feeds on nurse eggs will often reach a hatching size much larger or smaller than the mean for its species. In contrast, an embryo is likely to reach a standard size when its entire yolk supply is enclosed within its egg membrane (Fioroni, 1966). A variable hatching size may be an appreciable disadvantage (for example, if there is an optimum hatching size; Smith and Fretwell, 1974). Therefore, the factors leading to hatching size variations are likely to be of substantial importance to the snails.

An individual embryo can attain a relatively large hatching size only if it is able to acquire more than the average number of nurse eggs during its feeding period. An unequal distribution of nurse eggs can be obtained by three means. First, nurse-egg supplies may vary from egg capsule to egg capsule; when one capsule is more well-stocked with nurse eggs than others are, each of its embryos will acquire more than the average nurse egg supply. Second, some capsules may contain many more embryos than others even though all capsules are about equally well stocked with nurse eggs. Third, some embryos within each capsule may obtain more than their share of the nurse egg supply, thus attaining large size at the expense of (*e.g.*, out-competing) their capsulemates. The present research was initiated to determine which of these means is most important for two Eastern Pacific rocky shore snails, *Thais emarginata* and *Acanthina spirata* (Prosobranchia: Muricidae).

Materials and Methods

To obtain egg and embryo counts, capsules and adults of *Thais emarginata* were collected near Friday Harbor, San Juan Island, Washington, and from Dillon Beach (Bodega Bay) and Tomales Bay (Miller Park), Marin County, California; and capsules and adults of *Acanthina spirata* were collected from Tomales Bay. Females deposited capsules in the running water aquaria in both Washington (Friday Harbor Laboratories) and California (Pacific Marine Station). Field and laboratory capsules were kept in the aquaria until analyses were complete.

To obtain hatching sizes, capsules were isolated in small, closed containers during the hatching period. Hatchlings of *T. emarginata* were obtained unsystematically at Friday Harbor but all individuals of both species that hatched in the containers at Dillon Beach were measured.

Actual hatching sizes can be obtained only at the time of hatching. To obtain large samples on demand, an alternative measure was utilized—the sizes of late

[1] Contribution Number 53 from the Pacific Marine Station, University of the Pacific, Dillon Beach, California 94929.

embryos (embryos that withdraw fully into their protoconchs). In California, the average late embryo of *T. emarginata* reached a shell length (1.195 mm; N, 133; s.d., 0.176) nearly the same as that of a newly hatched snail (1.182 mm; N, 27; s.d., 0.141). No hatchlings were smaller than 0.9 mm (although some late embryos were), and 0.9 mm is accepted as a minimum hatching size. Late embryos of *A. spirata* are smaller (0.635 mm; N, 14; s.d., 0.029) than newly hatched snails (0.671 mm; N, 26; s.d., 0.058). Late embryo sizes cannot be equated precisely with hatching sizes, but they will be used as convenient and reasonable approximations.

Nurse-egg rations for *A. spirata* were obtained by comparing counts taken at cleavage stages (eggs + embryos) with counts of late embryos (embryos only). Larger capsules generally contain more embryos than smaller capsules (Spight, Birkeland and Lyons, 1974), and therefore, one must allow for capsule size differences when making comparisons. To do this, a relationship between capsule size and egg or embryo count was estimated for each sample of capsules. Linear relationships were obtained by regressing logarithms of counts on logarithms of capsule lengths (capsule body length, excluding stem). The counts included small and aberrant embryos, but data for capsules with excess nurse eggs were omitted (these capsules presumably did not have full complements of embryos).

Egg counts of *T. emarginata* were not made, but were instead estimated from late embryo sizes. The volume attained by an embryo was assumed to be proportional to the volume of yolk it consumed. A "volume index" proportional to the initial yolk volume was obtained for each capsule by adding the logarithmic mean embryo size to the logarithm of the number of embryos. The necessary data (sizes of all embryos) were obtained for twelve normal capsules and ten capsules with excess nurse eggs.

Results

Variability of hatching size

Newly hatched *Thais emarginata* from Friday Harbor had shell lengths of 1.55 to 1.80 mm; ones from Tomales Bay were 0.87 to 1.38 mm long (mean, 1.182 mm; s.d., 0.141; N, 27); and ones from Dillon Beach were 1.00 to 1.55 mm long (mean, 1.330 mm; s.d., 0.176; N, 10). Newly hatched *Acanthina spirata* from Tomales Bay were 0.55 to 0.75 mm long (mean, 0.67 mm; s.d., 0.058; N, 26).

Nurse-egg rations: Acanthina

All eggs of *Acanthina spirata* form gastrulae but some do not develop further, and the arrested eggs are eaten by the embryos. Some capsules from Tomales Bay (July 1973) contained 40–140 eggs in early cleavage stages, while other capsules contained 17–46 late embryos (Fig. 1). The mean number of late embryos (29.6; s.d., 7.84; N, 13) was subtracted from the mean number of eggs (79.0; s.d., 26.39; N, 11) to obtain the number of nurse eggs (on average, 1.67 nurse eggs per embryo; filled symbols of Fig. 1).

Both egg and embryo counts increase in proportion to capsule size (Fig. 1). To quantify the significance of the increase, coefficients of determination (r^2) were

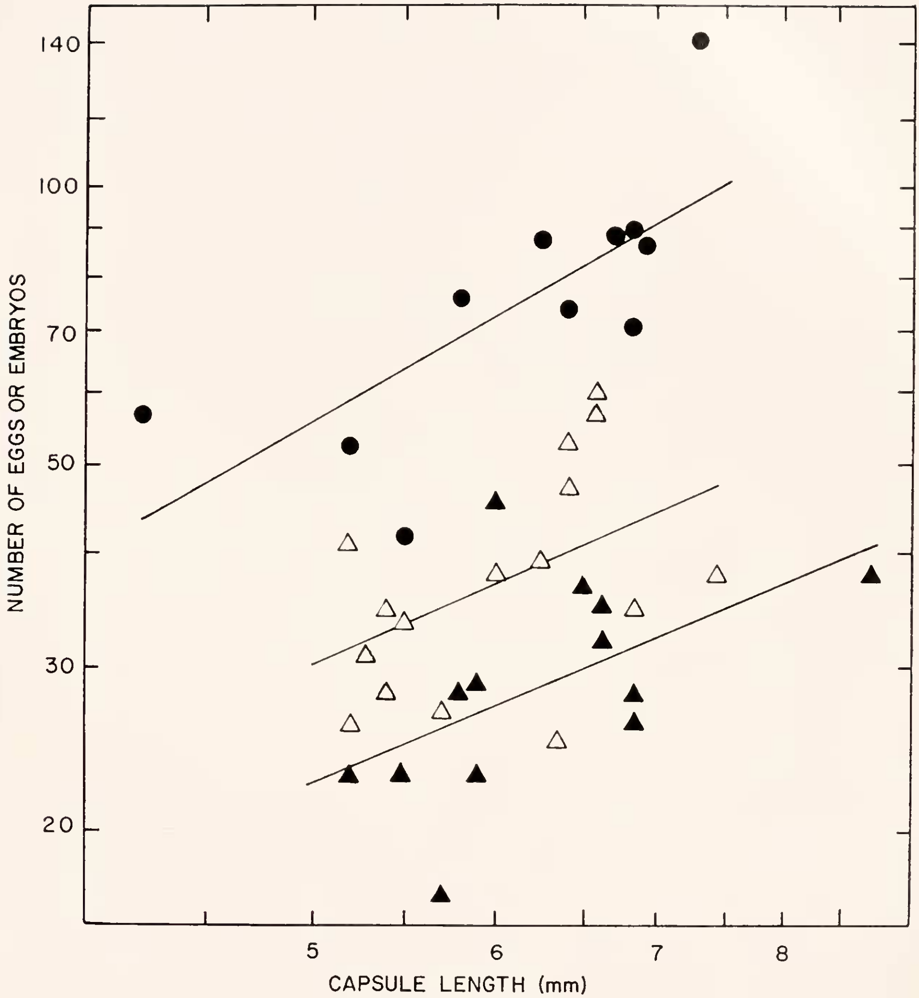

FIGURE 1. Contents of capsules of *Acanthina spirata* that were collected from the field (Tomales Bay, California, filled symbols), or were deposited in the laboratory (open symbols). Capsules containing egg stages (embryos plus nurse eggs) are indicated with circles, and capsules containing late embryos are indicated with triangles. Least-squares regression lines are plotted. Note that both scales are logarithmic.

calculated for these paired variables (Table I). Capsule length accounts for 55% of the variations in egg counts (r^2, 0.55). However, capsule size accounts for only 25% to 28% of the variations in embryo counts.

Several capsules were deposited in the laboratory, and these contained more embryos (38.4; s.d., 10.88; N, 16) than capsules from the field (Fig. 1). The late embryos in these capsules were also significantly smaller (mean shell length, 0.635 mm; s.d., 0.029; N, 14 embryos) than those found in capsules from Tomales Bay (mean, 0.699 mm; s.d., 0.049; N, 24; $F_{1,36} = 18.94$, $P < 0.001$).

TABLE I

Least-squares regression of logarithms of the number of embryos (Y) on logarithms of capsule length (X, mm). Capsules were collected in the field (F) in California and Washington, or were deposited in running-water laboratory aquaria (L). Egg counts (last line only) include both nurse eggs and embryos. All other counts include only late veligers. The total for Thais emarginata *includes four capsules collected at Dillon Beach.*

Source	Relationship	Number of capsules	Coefficient of determination	Mean square error	$F_{1,N-2}$	$\bar{X}$
	Thais emarginata					
Tomales Bay (F)	Y = 2.2590X − 0.7168	14	0.210	0.0431	3.1927ns	0.8362
Tomales Bay (L)	Y = 1.5110X − 0.0402	19	0.254	0.0252	5.7741*	0.7818
Study Strip	Y = 2.3473X − 0.8007	17	0.398	0.0215	9.9232**	0.8076
Shady Cove	Y = 2.5889X − 0.8506	9	0.087	0.0180	0.6656ns	0.7787
Total	Y = 1.6360X − 0.1683	63	0.202	0.0295	15.4101**	0.7989
	Acanthina spirata					
Veligers (F)	Y = 1.0619X + 0.6115	13	0.282	0.0105	4.9200ns	0.7967
Veligers (L)	Y = 1.2541X + 0.5934	16	0.250	0.0113	4.6692*	0.7775
Eggs	Y = 1.4714X + 0.7210	11	0.546	0.0100	10.8230**	0.7855

* $P < 0.05$; ** $P < 0.01$; ns, not significant.

Based on the egg counts for the field capsules, each laboratory embryo had 1.06 nurse eggs.

Nurse-egg rations: Thais

Large capsules of *Thais emarginata* generally contain more nurse eggs and more embryos than smaller ones. Capsule length (X) accounts for 54% of the variations in egg content (Y), when the volume index is used as a measure of egg content (nurse eggs + embryos). The linear regression between these two variables is: Y = 2.3513 X + 2.3082, for twelve capsules. Embryo counts (Fig. 2) are more variable; capsule length accounts for only about 20% of the variations in embryo count, and a unique relationship was obtained for each of the samples (Table I).

When embryo counts are highly variable, occasional large capsules will contain a few embryos and many nurse eggs, while occasional small capsules will contain many embryos and few nurse eggs. Maximum and minimum counts were examined to determine likely limits to nurse egg rations. Maximum counts increase steadily with capsule size. Among the capsules opened, 15 were smaller than 5.6 mm and none contained more than 20 embryos, while 85 capsules were longer than 5.6 mm and 18 of them contained 21 to 33 embryos. Within a length range of 4.3 to 8.2 mm, only capsules 6.2 to 7.7 mm contained 30 or more embryos, and the 7.7 mm capsule contained the maximum number of embryos observed (33). On the other hand, minimum counts do not increase with capsule size. In total, 11 capsules contained only 1 to 4 embryos, and 6 of these were larger than the average (6.3 mm; s.d., 0.77; N, 63). Therefore, the same minimum embryo count is common to capsules of all sizes.

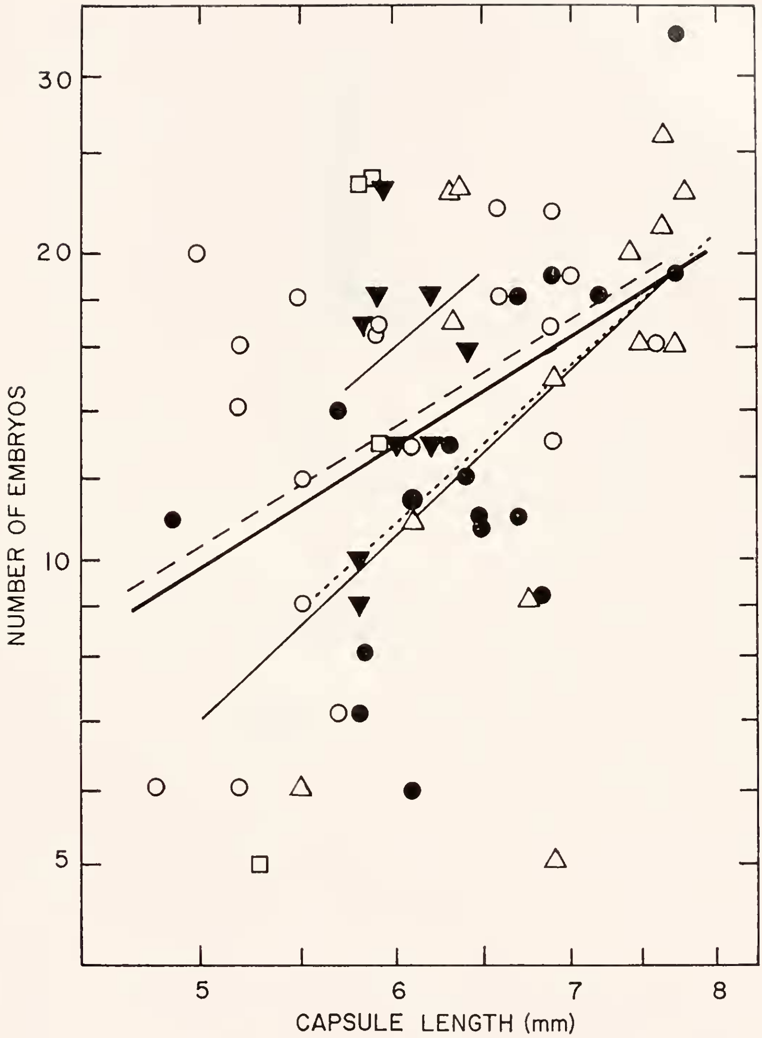

FIGURE 2. Contents (number of late embryos) of capsules of *Thais emarginata* that were collected in Washington (filled symbols) and California (open symbols). Washington capsules were obtained at two localities, Shady Cove (inverted triangles and upper solid line) and Study Strip (circles and lower solid line; see Spight, 1974). California capsules were obtained at Tomales Bay (field capsules, triangles and dotted line; laboratory capsules, circles and dashed line) and at Dillon Beach (squares). The heavy line in the center is a least-squares regression including all capsules.

Hatching size and number of capsulemates

Embryos have usually eaten all their nurse eggs long before the developmental period ends. However, some nurse eggs are left over when capsules of *T. emarginata* contain only one to four embryos. The volume indices (Y) for ten

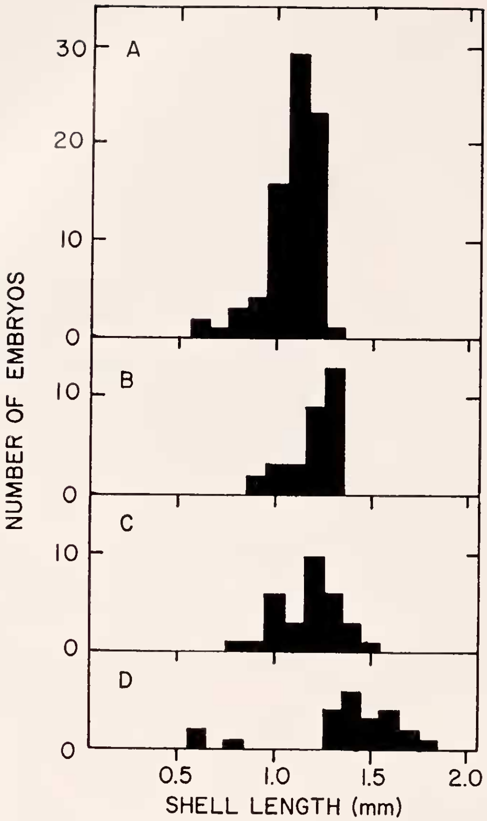

FIGURE 3. Effect of crowding on sizes reached by embryos of *Thais emarginata*. Capsules in A contain 17–23 embryos; capsules in B contain 14–16 embryos; capsules in C contain 6–11 embryos; and capsules in D contain 1–4 embryos (and excess nurse eggs). In a capsule containing four embryos, one embryo reached 3.02 mm; its size is not plotted.

such capsules are highly correlated (r^2, 0.90) with the number of embryos (X), indicating that all embryos eat about the same number of nurse eggs when nurse eggs are present in excess. With the relationship between these variables, $Y = 0.2066X + 2.9683$, it can be shown that the nurse egg supplies of the smallest capsules can be consumed by only four embryos, and six embryos can consume the supplies found in the largest capsules.

Since nurse egg supplies vary less from capsule to capsule than embryo counts, a typical embryo's nurse-egg ration will be more closely related to the number of capsulemates than to capsule size. In the capsule sample available, embryo sizes (Y, mean of the logarithms of shell lengths in mm) are highly correlated (r is −0.69) with embryo counts (number per capsule, X). The linear regression between these variables is $Y = 3.1463 - 0.0058\ X$, for twelve capsules.

Embryo size distributions, as well as mean sizes, change regularly as the number of embryos per capsule decreases (Fig. 3). When 17–23 embryos share a capsule, the distribution is sharply curtailed to the right and has a small tail to the left (Fig. 3A). Most embryos reach about the same size. Only 11 of 79 fall outside the narrow range, 1.0–1.2 mm; of these, six are smaller than the minimum hatching size of 0.9 mm, and only one has reached 1.3 mm. When 14–16 embryos share a capsule, the distribution is still curtailed to the right, but the left-hand tail contains a larger proportion of the embryos (Fig. 3B). The modal size increases to 1.3 mm, but no embryos have reached 1.4 mm (all are larger than 0.9 mm). With only 6–11 embryos, sizes form a nearly symmetrical distribution (Fig. 3C). Some embryos reach 1.5 and 1.8 mm, and others fail to reach 0.9 mm. When only 1–4 embryos share a capsule, the size distribution is nearly a mirror image of those for the crowded capsules. The distribution tails off to the right, and except for the three very small embryos, is sharply curtailed to the left (Fig. 3D).

Discussion

When a species provides nurse eggs to its offspring, some embryos typically reach much larger sizes than others. For example, hatching sizes range from 0.54 to 1.83 mm for *Murex virgineus* (Natarajan, 1957), 0.3 to 2.5 mm for *Thais lapillus* (Risbec, 1937), 3.5 to 8.5 mm for *Sipho islandicus* (Thorson, 1935), and 0.7 to 1.8 for *Murex brandaris* (the most variable of the species studied by Fioroni, 1966). Embryos of *Thais emarginata* and *Acanthina spirata* are provided with nurse eggs and reach variable hatching sizes. *T. emarginata* hatches as small as 0.9 mm and as large as 1.8 mm, a range of +44% to −31% about the mean shell length, and an 800% variation in weight. Hatching sizes for *Acanthina spirata* vary from +15% to −16% about the mean size, a range typical for species without nurse eggs (Fioroni, 1966).

An embryo must acquire more than the average nurse-egg supply to become larger than average. If excess nurse eggs are available, these will be eaten readily; embryos are usually capable of consuming several times the normal supply. For example, embryos of *Murex quadrifrons* and *Sipho curtis* will consume two to three times their usual rations (Knudsen, 1950; Thorson, 1935). The largest capsules of *T. emarginata* contain only enough nurse eggs to satiate six embryos. Since capsules rarely contain as few as six embryos (Fig. 2), typical embryos would eat many more nurse eggs than they get.

Since nurse eggs are in short supply, competition among capsulemates may affect hatching sizes. An embryo that begins feeding early and feeds rapidly will acquire more nurse eggs than a capsulemate that begins feeding late and feeds slowly. The size distributions for *Thais emarginata* show that some embryos acquire many more nurse eggs than their capsulemates. Embryos as small as 0.8 mm were found together with others as large as 1.8 mm after all nurse eggs had been eaten. However, large size differences among capsulemates are not typical (Fig. 3). When many embryos share a food supply, most reach about the same size; intracapsular size ranges in crowded capsules are as small as 1.0–1.2 mm. If competitive ability affected hatching size appreciably, then capsulemates would tend to be most dissimilar when food supplies are smallest (*e.g.*, in the most crowded capsules), and all embryos would tend to reach the same size when nurse eggs are present in excess. However, exactly the opposite is observed; embryo sizes are most varied when nurse eggs are present in excess (Fig. 3D; note the gradual increase in coefficient of variation of the mean: 13% for 17–23 embryos; 11% for 14–16 embryos; 16% for 6–11 embryos; and 23% for 1–4 embryos, excluding the three very small embryos of Fig. 3D). Therefore, competitive ability does not play a major role in generating hatching size differences among embryos of *T. emarginata*.

If competitive ability does not determine how nurse eggs are allocated, then nurse-egg rations must vary from capsule to capsule. The average embryo is substantially larger in some capsules than in others (Fig. 3). Therefore, nurse-egg rations are substantially larger than the average in some capsules.

An embryo will have a large nurse-egg ration if its capsule contains more than the usual number of nurse eggs, or, alternatively, if its capsule contains fewer than the usual number of embryos. Total egg counts (embryos plus nurse eggs) do vary from capsule to capsule. However, the counts are closely related to capsule size (Fig. 1); for *Acanthina spirata* and *Thais emarginata*, capsule size accounts for 54–55% of the variations in total egg count. Similar correlations between total egg counts and capsule sizes are observed for species without nurse eggs (five species: r^2 is 0.55 to 0.94, except *Eupleura caudata*, in which r^2 is 0.22; Spight, 1972; Spight, Birkeland and Lyons, 1974). Embryo counts are less predictable. Although small capsules seldom contain many embryos, some of the largest capsules contain only one or two embryos, and, overall, correlations between capsule size and embryo count are only marginally significant (Table I). Since embryo counts vary erratically from capsule to capsule, and nurse egg counts do not, embryos in some capsules will share a nurse egg supply with many capsulemates, while embryos in other capsules will share with few capsulemates. Each of the latter embryos will obtain a relatively large nurse egg ration and reach a large hatching size.

These data demonstrate that hatching sizes are variable for nurse-egg feeders because embryos are distributed erratically among capsules. Further work will be required to determine why embryos are distributed erratically and what advantages nurse-egg feeding provides to compensate for the disadvantage of a variable hatching size.

I thank Dr. R. H. Fernald for use of the facilities at Friday Harbor Laboratories, and Dr. S. Obrebski for facilities at Pacific Marine Station. Comments by D. L. West, R. Strathmann, and A. Lyons are greatly appreciated. Research was supported by NSF Grant GB 6518 X to the University of Washington, GB 3386 to Friday Harbor Laboratories and GA 25349 to R. T. Paine. Publication was supported by Woodward-Clyde Consultants.

SUMMARY

Some embryos of *Thais emarginata* attain twice the shell length of others by the time of hatching (0.9 to 1.8 mm, +44% to −31%, about the mean). Larger hatchlings must have acquired more nurse eggs than smaller ones. Embryos tend to reach the same size when nurse eggs are scarce (in crowded capsules), and size differences are most apparent when nurse eggs are present in excess. Therefore, competition among embryos contributes little to hatching size differences. Embryos are distributed haphazardly among capsules; some capsules contain only one embryo which becomes large, while others contain as many as 33 embryos, each of which

remains small. Nurse eggs are distributed more regularly. Embryos are also distributed haphazardly among capsules of *Acanthina spirata*. Each embryo of *A. spirata* has on average only 1.67 nurse eggs, and hatching size is correspondingly less variable (0.55–0.75 mm, +15% to −16%, about the mean). These nurse-egg feeders reach variable hatching sizes because some embryos share their yolk supplies with many more capsulemates than others.

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